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Imatinib has become the standard primary approach for the treatment of a newly diagnosed chronic phase myeloid leukemia (CML) patient, recently. Complete cytogenetic response is achieved on the majority of patients under treatment, but the disease remains in molecular level and after discontinuation of the drug, relapses occur inevitably. In imatinib era centers are referring only the patients, who has imatinib resistance or patients with high relapse risk. Secondly, the period from diagnosis to transplantation is an important nominator. In imatinib era CML patients will first undergo a trial of imatinib and according to the response they will be referred to the allogeneic transplant program. There is lack of data about the impact of delayed referral and emerging imatinib resistance on allogeneic transplant outcome. We have performed a case-matched control analysis in our CML (n: 173). CML patients who received imatinib before transplantation (n=20), were matched according EBMT (Gratwohl) score with imatinib naive ones (n=40). The median age at transplant in the imatinib group was 39 (19-57), gender F/M: 10/10, disease status 1st CP:13, 2nd CP: 4, AP: 2, BP: 1. The median total imatinib dose that was used until transplantation was 93gr (33-648), and imatinib therapy was discontinued at a median of 17, 5 (7-210) days prior to the transplantation. Only one of 20 patients was in molecular remission before transplantation. according to the EBMT score. The median age of 40 patients in imatinib naïve group was 34 years (14-53), gender F/M: 14/26, and disease status 1st CP:32, 2nd CP:2, AP: 3, BP: 3. The two groups were similar according to their age, sex, conditioning regimen, stem cell sources and Grathwohl scores. Engraftment for neutrophil $>0{,}5\times10^9$/L, and platelet $>20\times10^9$/L was median 15 (13-20) days and 13 (10-32) days in the imatinib group while it is 16 (0-28) days and 15 (0-36) days in the imatinib naive group. The response to transplantation, transplantation related mortality, the incidence of acute and chronic GVHDH for each group were not significantly different. Moreover, no significant difference was determined between 2-year's disease free survival and overall survival rates (Table). Our single center analysis is in concordance with several recent reports. In this study, it is observed that imatinib use prior to AHSCT does not negatively affect the early transplant related outcome.

	STI (+) n: 20	STI (-) n: 40	p
Median age	39 (18-57)	34 (14-53)	0,160
Grathwohl score (1,2,3,4)	1/2/7/6/4	1/6/20/10/3	0,545
Time Diagnosis-Transplant (month)	16,9 (5,4-75,8)	11,6 (4,2-129,3)	0,076
Stem cell source (PB/BM)	15/5	30/10	
Conditioning regimen (Ablative/RIC)	10/10	28/12	0,161
Sex (Female/Male)	10/10	14/26	0,751
Engraftment	19/20	39/40	0,559
NEU>0.5×10e9/L	15 (13-20)	16 (0-28)	0,639
Transplantation-related mortality	3/20	6/40	1
Acute GvHD (II-IV)	3/19	17/40	0,076
Chronic GvHD	8/16	22/34	0,322
Relapse rate	3/20	14/40	0,136
2-year's disease free survival	% 55,4±%13,2	% 46,7±%7,9	0,595
2-year's overall survival	%71,5±13,4	% 59,4±%7,9	0,463

PB: Peripheral blood, BM: Bone morrow, RIC: Reduced intensity conditioning

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HUMAN HERPESVIRUS-6 ENCEPHALITIS FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Approximately 50% recipients of allo-HSCT develop human herpes virus-6 (HHV-6) viremia detectable by polymerase chain reaction amplification (HHV-6 PCR), but the clinical significance of this asymptomatic viremia is unclear. Five of 53(9.4%) patients who received Alemtuzumab (total dose, 40 mg) supported conditioning (CY-TBI= 3, BU-TBI=1, Flu-Mel=1, MUD =3, MMUD =1, MMRD=1) and Tacrolimus as GVHD prophylaxis subsequently developed HHV-6 encephalitis whilst receiving antiviral prophylaxis (Valciclovir =4 and Valganciclovir =1). Acute GVHD (grade II= 1, grade III =2, grade IV=2) preceded encephalitis and had necessitated, prednisone =5, Infliximab =1, Alemtuzumab =1, and Daclizumab =1. HSV-6 encephalitis became apparent at 41-103 days (median 60 days) presenting with confusion (n=5), amnesia (n= 3) and seizures (n=2). MRI revealed nonspecific white matter changes in 4 and a non enhancing medial temporal lobe lesion in one of the patients. CSF protein was elevated in 4 patients (table-1); CSF-pleocytosis was mild with a median of 3- lymphocytes/hpf. HSV-6 PCR on blood (plasma) revealed 100-22,500 (median 1200) DNA copies/ml. CSF PCR was positive in all 5 patients at 600-225,000 (median 4700) copies/ml). CSF HHV6 was several fold higher than plasma levels (table-1). EEG was nonspecific in all 5 patients. Intravenous administration of foscarnet resulted in neurological improvement at 8-13 (median 11) days and negative plasma PCR at 30-66 (median 50) days; recovery of short-term memory loss was more prolonged. In the patient with negative plasma PCR, CSF PCR became negative on 50th day of therapy. Four patients had complete neurological recovery; one patient (#2) had transient improvement before succumbing to multi-organ failure. We conclude that, HSV-6 encephalitis complicates approximately 10% of *in vivo* T cell depleted allo-HSCT. Poor yield of routine CSF, MRI and EEG examination calls for high index of suspicion and CSF examination for HHV-6 PCR. Prompt antiviral treatment with foscarnet appears effective.

Table-1: *HSV-6 Encephalitis following allo-HSCT*

Age/ Sex	Onset/ Day	Manifestation	HSV-6 CSF (copy/ml)	HSV-6 Blood (copy/ml)	Outcome	Other viral infections	Follow-up Months
46F	+41	Amnesia, confusion, seizure	4700	Negative	Complete resolution	Herpes zoster	Alive 12 months
66F	+103	Confusion, somnolence, disorganized speech	225,000	22,500	Transient improvement	CMV	Died day +147
41M	+60	Amnesia, confusion	Positive*	200	Complete resolution	None	Alive 36 months
39M	+35	Amnesia, confusion, tremor	4800	100	Complete	BK virus resolution	Alive 6 months
58F	+83	Seizure, confusion	600	2700	Complete resolution	None	Died day +120

Abbreviations: M- male, F- female, allo-HSCT- allogeneic hematopoietic stem cell transplant, MRI- Magnetic resonance imaging, HHV- Human herpes virus, Prot- Protein, Leu- Leukocytes, CSF- Cerebrospinal fluid, WM- white mater, * Quantitative PCR not available

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CD154:CD40 CO-STIMULATORY BLOCKADE AT PRIMARY BMT PROMOTES ALLOGENEIC ENGRAFTMENT IN SECONDARY BMT BY BLOCKING SENSITIZATION

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Introduction: The morbidity associated with ablative conditioning has prevented the widespread application of bone marrow (BM) transplantation (BMT) to treat autoimmune diseases and hemoglobinopathies. To reduce the risk-benefit ratio of establishing chimerism, less-toxic approaches for conditioning recipients have been pursued. As one approaches the threshold for conditioning, failure of durable chimerism occurs with an increasing frequency. In the present studies, we evaluated the effect of non-ablative conditioning regimens on sensitization after graft failure and BM re-transplantation. **Materials and Methods:** Recipients were infused with MHC-disparate BMC in the context of costimulatory molecule blockade. In the primary BMT, B6 (H-2^b) mice were treated with anti-αβ-TCR mAb (day-3) and/or anti-CD154 (day0 and day+3), but without any total body irradiation (TBI), then transplanted with 15×10^6 allogeneic BALB/c (H-2^d) BM cells. Secondary BMT was performed 5-7 weeks after primary BMT with 950 cGy TBI and 15×10^6 BALB/c BM cells. **Results:** As expected, engraftment did not occur in mice after primary BMT. All animals survived. After secondary BMT with 950 cGy TBI conditioning, engraftment occurred in 25% of mice treated with anti-αβTCR alone at primary BMT. In contrast, all mice engrafted after secondary BMT with initial treatment of anti-αβTCR plus anti-CD154, and 75% of mice engrafted with anti-CD154 treatment alone at the first BMT. Flow cytometry cross-match assay was performed to detect anti-donor Abs in the sera collected 4 weeks after the first BMT. The values are reported as mean fluorescence intensity (MFI). The Ab titers were 4.9±2.6 MFI in mice treated with anti-CD154 and 4.0±0.4 in mice treated with both anti-αβTCR and anti-CD154 resembled the Ab level in naïve mice (3.4±0.5). Ab titers were significantly higher in mice treated with anti-αβTCR mAb alone (47.7±71.3; $P<0.05$) and the non-mAb treated group (123±34.7). **Conclusion:** These results suggest that CD154:CD40 co-stimulatory blockade used at the time of primary BMT promotes allogeneic engraftment in secondary BMT after engraftment failure. The mechanism is that anti-CD154 inhibits B cell activation and generation of alloantibody after primary BMT. Circulating anti-donor Abs are therefore the critical hurdle for the success of secondary BMT. These findings could have a significant impact on management of clinic recipients who have failed at primary BMT and require retransplantation.

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THE IMPACT OF EARLY ONSET OF HEMOPHAGOCYTOSIS AFTER TRANSPLANTATION ON THE OUTCOME OF ALLOGENIC STEM CELL TRANSPLANTATION

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[Introduction] Recently, the concept of early onset of hemophagocytosis after transplantation has been proposed, but the incidence, pathological findings and prognosis have not clarified. We analyzed how the early onset of hemophagocytosis after transplantation affects the outocome of allogenic stem cell transplantation.

[Patients and methods] Fifty-two patients received their transplants at the Nagoya First Red Cross Hospital from May 2005 to April 2006. Forty-five patients of the 52 patients received marrow aspiration in early stage of transplantation (until day30), and were included in the current study. Patients' ages were from 17 to 65 (median 42) years old. Forty patients were malignant diseases, and 5 patients were non-malignant diseases. Nineteen patients received reduced intensity stem cell transplantation (RIST) regimen, and 36 patients received conventional regimen. Thirty-two patients received BMT, 8 patients received PBSCT, and 5 patients did CBT. Clots of bone marrow were retrospectively analyzed by a pathologist, and they were divided into three groups according to the intensity of pathological findings of hemophagocytosis as group A (no hemophagocytosis), group B (mild hemophagocytosis) , and group C (severe hemophagocytosis). We compare three groups with the clinical parameters.

[Results] Twenty-two patients were included in group A (49%), 13 patients in group B (29%), and 10 patients in group C (22%). The average of maximum T-bilirubin in early stage of transplantation (until day 30) was 1.60 mg/dl (group A), 2.68 mg/dl (group B), and 6.49 mg/dl (group C).There was significant difference between A group to C group ($p<0.01$). Similarly, the average of maximum creatinine was 0.78 mg/dl (group A), 0.75 mg/dl (group B), and 1.26 mg/dl (group C). There was significant difference between A group and B group to C group ($p<0.01$). Disease free survival until day 100 was 4.5% (group A), 7.7% (group B) and 40% (group C) ($p=0.02$).

[Conclusion] It is supposed that the occurrence of hemophagocytosis until day 30 were well associated with elevation of T-bilirubin and creatinine, and early mortality. To confirm a clinical significance of hemophagocytosis, farther studies are needed.

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ALTERATION OF NK TOLERANCE EARLY POST-ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION RESULTS IN NK ALLOREACTIVITY DRIVEN BY LACK OF RECIPIENT HLA LIGAND FOR DONOR INHIBITORY KIR

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Donor NK alloreactivity can play a significant role in hematopoietic cell transplantation (HCT) outcome by decreasing leukemia relapse and increasing survival. How NK cells in HCT behave through killer Ig-like receptor (KIR) recognition of target cell HLA has been the subject of discussion, with evidence supporting a "missing self HLA" mechanism, relevant to KIR ligand incompatible (HLA-mismatched) HCT, and other studies supporting a "missing HLA ligand" mechanism, relevant to both HLA-matched and mismatched HCT. We propose that in HCT these two mechanisms need not be mutually exclusive. Using 6-color flow cytometric analysis of intracellular IFN-γ production, we evaluated the response of inhibitory KIR-expressing NK subsets following activation with 721.221, a target cell line deficient in class I expression, or with 721.221 transfectants expressing the KIR ligands HLA-Cw3, -Cw4, or -Bw4. NK cells from 10 KIR haplotype-A homozygous individuals and from 7 T-cell depleted HCT donor-recipient pairs were evaluated. In normal individuals, mature NK cells expressing inhibitory KIR specific for self-HLA were significantly more responsive to target cells lacking KIR ligand than NK cells expressing inhibitory KIR for non-self HLA ($P<0.001$), consistent with "missing self" behavior. NK cells expressing KIR2DL3, 2DL1, and 3DL1 all demonstrated similar response patterns, which were predictable based on the HLA background of the individual. However, NK cells from HCT patients displayed significantly different response patterns to the same target cells: compared to the NK subsets in the donor, the recipient NK response to lack of HLA ligand, both *self* and *non-self*, was higher at days 15-60 ($P<0.001$), decreasing to donor levels by day 100. Indeed, the response of NK cells specific for non-self HLA early post-HCT was equivalent to the response of NK cells specific for self-HLA in a normal individual. Thus, while the mature NK cell achieves tolerance by preferentially endowing functional competence to inhibitory KIR specific for self-HLA ("missing self"), the stem cell-derived NK cell post-HCT achieves this self-tolerance only after transitioning from a state where all inhibitory KIR receptors are capable of recognizing lack of ligand ("missing ligand"). These results indicate that NK alloreactivity in HCT is driven by both mechanisms and that HLA-identical HCT recipients benefit from NK alloreactivity early post-HCT due to lack of HLA ligand for donor inhibitory KIR.

GVH/GVL

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HUMAN TREGS INDUCED BY ECDI-COUPLED APC FOR THE SUPPRESSION OF TRANSPLANTATION ALLORESPONSES

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